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Development of a general purpose

Disinfectant for Military Use

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A survey of the large number of chemicals and formulations available on the market today indicated that only a few compounds would be suitable for a dry type, concentrated disinfectant which will fulfill a variety of essential military requirements. The first essential requirement for such a disinfectant is that the formulation must be dry and in a concentrated form to save space and to make for easier handling. The primary use for this type of disinfectant is for the disinfection of the contents of field latrine buckets. Although it is expected that such a dry type formulation will have a wide military application as a general purpose disinfectant, the object of this specific investigation is limited to the development of a concentrated formulation suitable as a field latrine bucket disinfectant. The results of the application of this dry type formulation as a general purpose disinfectant will be reported at a later date.

The disinfectant must satisfy, at least to a reasonable extent, all or nearly all of the requirements for the various conditions of military use; speed of disinfection is probably not of primary importance, although it should be accomplished within a reasonable time, i.e. within about five minutes; the temperature for most uses will probably be about 20°C, though it is possible that five to 10°C might be encountered under unusual conditions of use; the product must retain its disinfectant capacity in the presence of a high organic load; the product must be reasonably stable during shipment and storage as well as under the conditions of and during the time of use; the product must not be excessively corrosive to metals with which it will come into contact during the time of use; the product should be reasonably safe from toxicity and irritation under the conditions of use and during normal handling. In the present investigation, these military requirements have been considered with respect to the selection of the formulation which best meets these requirements.

Experimental Methods

Total nitrogen in stool: Since human excreta contain an unusually high concentration of organic matter largely as protein, nitrogen analyses by the Kjeldahl method (1) were made both on the intact stool and on the liquid material from latrine buckets.

Test cultures: Escherichia coli, American Type Culture Collection (ATCC) 26, *Salmonella typhosa,* ATCC 6539, and *Micrococcus pyogenes* var. aureus, ATCC 6538 (FDA 209) were used as test cultures in the modified (2) phenol coefficient (3) tests. *Escherichia coli* used for most of the screening tests was found to be more resistant to the germicides tested than the *Salmonella typhosa.*

Salmonella choleraesuis, ATCC 10708, and *Micrococcus pyogenes* were also used for evaluation of a few selected formulations by the use-dilution confirmation test (4).

The test cultures were maintained on Bacto Stock Culture Agar following the procedure recommended in the phenol coefficient test procedure.

Bactericidal test procedures: Screening tests were carried out by the Cade and Halvorson (2) modification of the phenol coefficient test (3). In the Cade and Halvorson procedure, the germicide-culture mixture is transferred to a solid medium instead of to a liquid medium as in the phenol coefficient method.

The temperature of the test solution was maintained within plus or minus 0.5° of the stated temperature in a thermostatically controlled water bath. (Unless otherwise indicated, the temperature was 20°C). Exposure times were two, four, six and eight minutes, except where otherwise noted. "Tween" asolectin nutrient agar, made by solidifying the A.O.A.C. phenol coefficient test medium with 1.5 per cent agar, was used for the subculture medium. In other details, the phenol coefficient test procedure was strictly adhered to. A zero plate count by the Cade and Halvorson method was interpreted to correspond to "no growth" in phenol coefficient test.

Field test was carried out on a selected formulation by testing its ability to decontaminate the natural bacterial flora of the stool.

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Survival of "total bacteria" was determined by "plating out" a loopful (4 mm. I.D., 26 gauge platinum loop) of sample on tryptone glucose extract agar and the survival of coliforms was determined by inoculating simultaneously eosin methylene blue agar and lactose broth medium.

Buffers: The effectiveness of many bactericides is a function of the pH of its solution. Phenolic derivatives are reported to have their maximum bactericidal effect when one-half of the molecules are in the salt form and one-half in the acid form (5). Where it is desired to study the effect of pH to determine the optimal range, the formulations were buffered with McIlvaine's citrate-phosphate buffer (6). At the pH range near the pKa value phenolic compounds themselves had some buffering action. All pH measurements were made with a glass electrode using Coleman's Certified Buffers to standardize the pH meter in the operating range.

Shelf life test: Because the product must be stable and not lose its effectiveness during normal periods and conditions of storage, samples of a mixture of 75 per cent sodium - chloro - o - phenyl phenate and 25 per cent sodium-o-phenyl phenate (hereinafter referred to as the proposed disinfectant), were stored in a thermostatically controlled oven at 50°C and tested for residual bactericidal activity after varying periods up to and including 540 days. The product was subjected to this relatively high temperature because it is known that such temperatures are sometimes recorded in the carriers which used to transport these goods (7); furthermore, this temperature will give accelerated aging and may possibly enable one to predict in a short time what degradation might take place on longer storage at "room" temperatures. Additional stability tests which include subjecting the product to other temperature and humidity conditions are contemplated in order to obtain additional information on the ability of this product to withstand extremes of climatic conditions.

Corrosion studies: Corrosion characteristics of the use solutions (0.2 to 0.5 per cent in distilled water) toward cold rolled steel and galvanized iron were determined by static immersion of 2 x 2 inch squares of the test materials for 21 hours at 37°C. The losses in weight of the test pieces were recorded and then calculated in terms of the loss in mgs./sq.cm. of total surface/24 hours. Corrosion tests on final proposed formulation showed some effect on galvanized metal, but essentially none against cold rolled steel. No further mention of this phase of the work will be made.

Toxicity and skin irritation tests: Intended use of the dry type disinfectant did not warrant extended skin irritation and toxicity tests. Day to day handling of the best phenolic disinfectant, (75 per cent sodium-chloro-o-phenyl phenate and 25 per cent sodium-o-phenyl phenate) and its solutions indicated no particular hazard. Hand soaking tests indicated that one gets only slight reddening of the skin, but definite necrosis on prolonged exposures. Military toxicity clearance for 15,000 ppm aqueous solution of the proposed disinfectant has been approved for initial use in 14 quart latrine buckets. It is considered that a latrine bucket of 14 quarts capacity will be emptied after it is half full and under such use conditions an initial charge will be so selected as to leave a final concentration of 2,000 ppm of the disinfectant.

Table I. Kjeldahl Determinations on the Stool and Liquid Portions of the Latrine Bucket

	%N		% Protein (%N x 6.25)	
Sample	Stool Sample	Liquid Sample	Stool Sample	Liquid Sample
1	0.56	0.49	3.5	3.1
2	0.84	0.49	5.2	3.1
3	1.26	0.49	7.9	3.1
4	1.67	0.485	10.4	3.1
5	0.86	0.485	5.4	3.1
6	1.65		10.3	3.1
Average	1.14	0.49	7.1	3.1

Results

Results of the Kjeldahl nitrogen determinations on stool samples summarized in Table I indicate a wide variation in the nitrogen content from one individual to another as has been reported by others (8). This variation is probably a function of age, the amount and kind of food intake and the amount of exercise, as well as inherent differences in the metabolism of the individual.

The data indicate that intact stool contains about seven per cent protein, while the liquid contains about three per cent protein. Assuming that there is about equal weight of liquid and solid, the average protein contents of the waste in the latrine bucket is about 5.0 per cent protein.

These figures would indicate that about five per cent protein ought to be added to the test solution in a germicidal performance test. In the majority of the tests reported the organic matter load was supplied by adding 5.0 per cent peptone to the disinfectant solution.

Various types of compounds were selected for screening based on our previous experience and indications in the literature; some of these were expected to be of limited value, but were tested in order to obtain comparative and more complete information, since not all of the tests reported in the literature were carried out under the same conditions.

Comparative performance of several of these selected compounds

from each of the several classes of compounds (halogen, quaternary ammonium and phenolic compounds) are summarized in Tables II, III and IV. These compounds are believed to be the outstanding ones in each category.

The results summarized in Table II indicate that chloromelamine (Halogen II) and iodine formulation (Halogen IV) will give zero counts in about two minutes at a concentration of 2,000 ppm available halogen, in the presence of five per cent peptone. These tests were carried out within five to ten minutes after the addition of peptone and consequently do not show the effect of aging of the solution on its bactericidal activity. Other studies indicate that the stability of these compounds in solution is far too poor for the use of disinfecting contents of field latrine buckets.

The data shown in Table III indicate that alkyl dimethylbenzyl ammonium chloride (Quat A) is adversely affected by 5.0 percent peptone. However, the presence of 1.0 percent of a suitable detergent mixture (containing a nonionic detergent and sodium tripolyphosphate) materially overcomes this deleterious effect of the peptone.

The addition of 0.75 percent linseed oil soap interferes with the bactericidal action of the quaternary to such an extent that zero counts are not obtained even after 15 minutes exposure time at a concentration of 1,000 ppm of the quaternary.

The purpose of adding soap to the disinfectant mixture was to stimulate possible use of the disinfectant with soap as a disinfectant-cleaner combination when used as a general purpose disinfectant.

Similar, but slightly poorer, results were obtained with di-isobutyl - phenoxy - ethoxy - ethyl - dimethyl-benzyl ammonium chloride (Quat B). Previous experience with a number of quaternaries indicates that alkyl (C_8H_{17} to $C_{18}H_{37}$) dimethyl benzyl ammonium chloride is one of the best quaternaries available with respect to water hardness tolerance and bactericidal spectrum.

Unquestionably several of the quaternaries have very excellent germicidal properties and are useful germicides, however, their use as a combined latrine bucket

Table II. Bacteriological Tests on Active-Halogen Compounds

Halogen** Formulation	pH of test solution	Percent concentration of formulation	PPM available halogen in solution	Plate counts* after exposure time shown (in minutes)			
				2.5	5	10	15
I. In 5% peptone solution	8.0-8.3	1	300	10000	10000	8000	5000
		0.5	150	10000	10000	10000	8000
		0.2	60	10000	10000	10000	8000
		0.1	30	10000	10000	10000	10000
II. In 5% peptone solution	3.0-3.5	1	2000	0	0	0	0
		0.5	1000	3000	11	0	0
		0.2	400	10000	10000	4000	1500
		0.1	200	10000	10000	10000	10000
				Plate counts after exposure time in minutes			
				2	4	6	8
III. Alone		4.0	2000	0	0	0	0
		2.0	1000	0	0	0	0
		1.0	500	0	0	0	0
		0.4	200	0	0	0	0
III. In 5% peptone solution	6.4-7.0	4.0	2000	10000	10000	10000	10000
		2.0	1000	10000	10000	10000	10000
		1.0	500	10000	10000	10000	10000
		0.4	200	10000	10000	10000	10000
IV. Alone		0.7	2000	0	0	0	0
		0.35	1000	0	0	0	0
		0.17	500	0	0	0	0
		0.07	200	0	0	0	0
IV. In 5% peptone solution	6.0-6.5	0.7	2000	0	0	0	0
		0.35	1000	250	500	300	150
		0.17	500	10000	10000	10000	10000
		0.07	200	10000	10000	10000	10000

Conditions of experiment: 1. Med. Temp. 20°C. 2. Organism *E. coli* (ATCC #26). 3. Inoculum: 1 ml 24 hr. broth culture/10 ml medication. 4. Organic matter: 5% peptone.

*A plate count of 1000 is equivalent to about 99.9% kill; a plate count of 100 equivalent to 99.99% kill etc.

**Formulations

Halogen I	Azochloramid — Buffer sale mixture	60%
	Alkyl aryl sulfonate 40% active	40%
Halogen II	Chlormelamine (chlorination product 1,3,5 triamino triazine)	21%
	Sodium dodecyl benzene sulfonate 85% active	12%
	Citric acid	53%
	Sodium dihydrogen phosphate (NaH_2PO_4)	14%
Halogen III	Iodine resublimed U.S.P.	5%
	Nonionic detergent (an alkyl aryl polyethylene glycol ether	5%
	Urea	90%
Halogen IV	Iodine resublimed	28.5%
	Potassium iodide	71.5%

Table III. Bacteriological Tests on Selected Quaternary Ammonium Compounds

Test Material	PPM of Quat in solution	pH of test solution	Plate counts[1] after exposure time (minutes) shown			
			2	4	6	8
Quat A[2]	200	7	0	0	0	0
	100		0	0	0	0
	50		2	2	1	0
	25		2410	350	94	43
Quat A plus 5% peptone[3]	5000	7	0	0	0	0
	2000		0	0	0	0
	1000		196	4	0	0
	500	10	10000	3000	350	33
Quat A plus 1% detergent[4] salts.						
No peptone	2000	10.0-10.2	0	0	0	0
	1000		0	0	0	0
	500		0	0	0	0
	200		0	0	0	0
Quat A plus 1% detergent salts + 5% peptone	2000	8-9	0	0	0	0
	1000		0	0	0	0
	500		0	0	0	0
	200(?)		400	572	6	1
			5	**10**	**15**	
Quart A plus 1% detergent salts + 0.75% linseed oil soap	1000		8000	4000	290	
	500		10000	5000	525	
	250		10000	5000	950	
Quat B[5] formulation alone	1500	10.0-12.2	0	0	0	0
	750		7	0	0	0
	300		25	4	0	0
	150		78	4	0	0
Quat B formulation plus 5% peptone	1500	8.7-7.6	2	0	0	0
	750		3000	2000	1200	500
	300		10000	10000	10000	10000
	150		10000	10000	10000	10000

Conditions of test — (same as in Table II): 1. A plate count of 1000 is equivalent to 99.9% kill; a plate count of 100 equivalent to 99.99% reduction etc. 2. Quat A is an alkyl dimethyl benzyl ammonium chloride wherein the alkyl group is C_8-C_{18} (principally C_{12}). 3. Peptone is Bacto Peptone from Difco Co., Detroit. Peptone was added to give final concentrations of 5%. 4. Detergent Salt: A mixture of compatible nonionic wetting agent and inorganic detergent salts of the following proportions: light ash 40 parts, sodium tripolyphosphate 30 parts, sodium bicarbonate 10 parts, sodium chloride 10 parts, nonionic detergent (an alkyl aryl polyethylene glycol ether) 5 parts. The quaternary solution and the detergent salt solution were mixed to give the desired concentration of each. 5. Quat B is a mixture of 15 parts di-isobutyl-phenoxy-ethoxy-ethyldimethyl-benzyl-ammonium-chloride-monohydrate, 5 parts of an alkyl aryl polyethylene glycol ether, 30 parts sodium tripolyphosphate, 40 parts light ash and 10 parts sodium bicarbonate.

Table IV. Effect of pH on the Bactericidal Activity of Substituted Phenols

Plate counts after times (in minutes) shown below

pH	ppm Phenol	Sodium chloro-o-phenyl phenate 2 min	4 min	6 min	8 min	Sodium 2-chloro-4-phenyl phenate 2 min	4 min	6 min	8 min	Sodium o-benzyl-o-chlorophenate 2 min	4 min	6 min	8 min
5.02	2000	0	0	0	0	27	2	1	0	0	0	0	0
	1000	0	0	0	0	68	6	5	0	0	0	0	0
	500	0	0	0	0	108	48	20	11	0	0	0	0
	200	9	0	0	0	1180	204	113	69	8	0	0	0
7.0	2000	0	0	0	0	1200	166	25	10	0	0	0	0
	1000	0	0	0	0	2800	460	141	43	0	0	0	0
	500	0	0	0	0	3100	1200	212	88	0	7	0	0
	200	3	0	0	0	4500	2800	664	332	1	15	0	0
9.0	2000	0	0	0	0	0	0	0	0	0	0	0	0
	1000	0	0	0	0	0	0	0	0	0	0	0	0
	500	0	0	0	0	0	0	0	0	0	0	0	0
	200	0	0	0	0	5400	2900	2100	1700	0	0	0	0
11.0	2000	0	0	0	0	331	6	0	0	0	0	0	0
	1000	1500	4	0	0	3000	1800	222	110	0	0	0	0
	500	4000	2000	2000	800	6500	3000	2800	2600	0	0	0	0
	200	6000	4000	3000	2500	104	5500	4000	2800	4000	1200	148	2

The pH of the sodium phenolate solution was adjusted with normal NaOH and H_3PO_4. McIlvane's Buffer was used for stabilizing the pH. Medication temp 20°C. Organism: E. coli (Atcc #26). Inoculum: 1.0 ml 24 hr culture/10 ml medication Organic matter: 5% peptone.

and general purpose disinfectant is limited for several reasons. Quaternary ammonium compounds as a whole show varying abilities to tolerate hardness. There are several quaternaries which, when formulated with properly selected sequestering agents and detergents salts, are not appreciably affected even by extremely hard water. However, even these are appreciably affected by some inorganic detergent salts. Moreover, quaternaries must not be used with soaps or synthetic anionic detergents.

Table IV is a summary of the tests on several selected phenolic derivatives. As a result of practical consideration of such factors as bactericidal activity, stability (i.e. shelf life as well as solution life), availability, costs, etc., o-benzyl-o-chlorophenol, chloro - o - phenylphenol and 2 - chloro - 4 - phenylphenol were selected for study from a number of phenolic compounds which are described in the literature (9), (10), (11), (12), (13), (14), (15), (16).

BACTERICIDAL action of many disinfectants is affected by pH, including the phenolic derivatives. A part, possibly a major part of this effect on phenolics is probably associated with the effect of the pH on the solubility of the compound. All three principal compounds studied, the chloro - o - phenylphenol, 2-chloro-4-phenylphenol and o-benzyl-p-chlorophenol are only slightly soluble in water. However, their corresponding sodium salts are very soluble. In all cases, solutions of their corresponding sodium salts were prepared, adjusted to the desired pH with sodium hydroxide, and buffered with McIlvane's citrate-phosphate buffer (pH 5.0, 7.0, 9.0 and 11.0).

A turbid solution resulted in all cases when the back-neutralization of the phenate ion was carried beyond the solubility of the free phenol. The resulting emulsion formed in the case of the 2-chloro-4-phenylphenol solution was unstable due to the tendency of the compound to crystallize readily out of solution. The chloro-o-phenylphenol and o-benzyl-p-chlorophenol solutions did not tend to crystallize; the melting point of these two compounds being less than -20°C and 45°C, respectively. 2-chloro-4-phenylphenol, on the other hand, has a melting point of 74°C. The commercial choloro-o-phenylphenol is an isomeric mixture of 4-chloro-2 - phenylphenol and 6 - chloro - 2 phenylphenol and was resolved on the basis of the differential solubilities of their calcium salts (17) and identified via their corresponding mono-nitro-derivaties (18), as well as checked by their infra-red spectograms against known samples.

Fatty acid soaps, anionic synthetic surface active agents, and nonionic alkyl aryl polyethylene glycol ether all have deleterious effects on bactericidal action of the phenolic derivatives tested. Among the various fatty acid soaps tested, coconut oil soaps had the least effect, soybean oil and cottonseed oil soaps had the greatest effect, and linseed oil soaps were intermediate. Alkyl aryl polyethylene glycol ether had greater effect than either the anionic synthetic detergents or the fatty acid soaps. Although the phenolics can be used in the presence of soaps and anionic synthetic detergents, provided the phenolics are used at a moderately high concentration, they are much more effective in the absence of such substances. The data indicate that phenolics and nonionic detergents of the type tested should not be used together. These data suggest, also, that it is not economical to use these phenolic materials with soaps (except coconut oil soap) in routine disinfection of latrine buckets. A close inspection of the data found in Table V indicates that a mixture consisting of 75 per cent sodium chloro-o-phenylphenate and 25 per cent sodium o-phenyl-phenate gives the most satisfactory result of all formulations tested.

Phenolic compounds are less affected by organic matter and by soaps (in these and other studies) than the quaternary ammonium compounds. On the other hand, extremely alkaline detergents such as caustic soda and sodium metasilicate are detrimental to the action of phenol. In the disinfection of field latrines, however, these strongly alkaline materials are not likely to be used. Weighing these differences, then, it is believed that phenolics have an advantage over the quaternaries for this particular use. This might not necessarily be true for all quaternaries and phenolics as a class, but rather on a basis of comparison of several of the compounds tested which are believed to be the best available in each group.

Tables VI, VII, and VIII summarize some further studies on the best formulation (75 per cent sodium chloro - o - phenyl - phenate and 25 per cent sodium o-phenyl-phenate). Table VI is a summary of studies on the disinfection of stool by the proposed disinfectant. At a concentration of 2,000 ppm of the proposed disinfectant, the liquid phase in the latrine bucket was found to be sterile after a five minute contact period. The intact stool, however, was not sterile even after a 60 minute contact period, although no coliform could be recovered from it. At a 1,000 ppm concentration, similar results were obtained. However, when the concentration of the germicide was lowered to 500 ppm the liquid phase was not sterile even after 45 minutes contact, although no coliform was recovered even at the 5 minute contact period. Water tem-

Table V. Effect of Added Peptone, Fatty Acid Soaps, and Other Detergents on the Bactericidal Properties of Various Phenolic Derivatives on Mixtures of Phenols

Test material	Composition of Formulation (% by weight) Sodium-o-phenyl phenate	Sodium trichloro-phenate (2,4,5)	Sodium chloro-e-phenyl phenate	Isomeric benzyl cresols	P-chloro meta-xylenol	Others	Builders	Inactivator	ppm Phenol required for zero count. Exposure times as shown (min.) 2	4	6	8
1.	25		75	.....				None	500	500	200	<200
								5.0% Peptone	>500	>500	500	500
								0.1% Nacconol NR	500	500	500	500
								0.2% Nacconol NR	1000	1000	500	500
								0.5% Ocean green	1000	1000	500	<500
								1.0% Ocean green	2000	1000	1000	500
								0.2% Coconut oil soap	<500	<500	<500	<500
								0.5% Coconut oil soap	1000	1000	500	500
								1.0% Coconut oil soap	1000	1000	500	500
								0.2% Soya Bean oil soap	2000	1000	1000	1000
								0.5% Soya Bean oil soap	5000	2000	2000	2000
								1.0% Soya Bean oil soap	5000	5000	5000	2000
								0.2% Cottonseed oil soap	2000	1000	1000	<1000
								0.5% Cottonseed oil soap	.5000	2000	2000	<2000
								1.0% Cottonseed oil soap	5000	5000	5000	2000
								0.2% Linseed oil soap	1000	1000	500	500
								0.5% Linseed oil soap	2000	2000	1000	<1000
								1.0% Linseed oil soap	5000	2000	2000	2000
								0.05% Igepal* C.A.	5000	5000	2000	2000
								0.10% Igepal* C.A.	>5000	>5000	>5000	5000
								0.20% Igepal* C. A.	>5000	>5000	>5000	>5000
2.		25	75	.....	.....			None	500	500	200	<200
								5.0% Peptone	500	500	200	<200
								0.5% Cottonseed oil soap	5000	5000	2000	2000
								1.0% Cottonseed oil soap	>5000	5000	5000	5000
								0.5% Soya Bean oil soap	5000	2000	2000	2000
								1.0% Soya Bean oil soap	>5000	5000	5000	5000
3.	25	25	50	.....	.....			None	500	500	500	500
4.	20		60	.....		20% Tri poly		5.0% Peptone	500	500	500	500
								None	<500	<500	<500	<500
								0.5% Cottonseed oil soap	5000	2000	2000	1000
								1.0% Cottonseed oil soap	5000	2000	2000	2000
								0.5% Cottonseed oil soap	1000	1000	1000	1000
								+1.0% Pretone	1000	1000	1000	1000
								2.0% Peptone	1000	1000	1000	1000
5.	12		48	.....	....			0.5% Cottonseed oil soap	2000	2000	1000	1000
						40% Tri-poly		1.0% Cottonseed oil soap	5000	2000	2000	2000
6.			50		50*Na bis (2 hydroxy-3,5,6-trichloro-phenyl methane)			0.5% Cottonseed oil soap	5000	5000	2000	2000
7.			50		50 Na bis (5 chloro-2-hydroxy-penyl methane)			0.5% Cottonseed oil soap	5000	5000	2000	2000
8.					Disinfectant latrine bucket 51D 377			0.5% Cottonseed oil soap	2000	2000	2000	2000
9.			50		50 Bisphenol mono-sodium salt			1.0% Cottonseed oil soap	5000	5000	2000	2000
					[Na bis (hydroxyphenyl propane]			0.5% Cottonseed oil soap	5000	5000	2000	2000
							Builders					
							Water Conditioners Soap					
10. (Tested at 37°C)	20		60				20% Versene**	None	<500	<500	<500	<500
								0.5% Cottonseed oil soap			2000	1000

(Table continued on following page)

Conditions of Experiment: 1. Medication Temp.: 20°C 2. Organism: E. coli (ATCC #26) 3. A plate count of 1000 is equivalent to about 99.9% kill, a plate count of 100 is equivalent to 99.99% kill, etc.

Notes: Versene: Tetrasodium ethylene diamine tetracetate Pyro: tetrasodium pyrophosphate Tripoly: sodium tripolyphosphate

*"Igepal" is registered trade name of Antara Chemicals, a Division of General Aniline & Film Corp., New York.
**A registered trade name of Dow Chemical Co., Midland, Mich.

Table V (Continued). Effect of Added Peptone, Fatty Acid Soaps, and other Detergents on the Bactericidal Properties of Various Phenolic Derivatives on Mixtures of Phenols

Test Material	Composition of Formulation (% by weight) Sodium-o-phenyl phenate	Sodium trichloro-phenate (2,4,5)	Sodium chloro-o-phenyl phenate	Isomeric benzyl cresols	P-chloro meta-xylenol	Others	Builders	Inactivator	ppm Phenol required for zero count. Exposure times as shown (min.)			
11. (Tested at 37°C)				36.7	6.12% NaOH 28.6% Pyro-28.6% Coconut oil soap			None	>250	>250	<250	<250
								300 ppm hard water	500	<500	<500	>500
								5% Peptone	2000	2000	2000	<2000
								0.5% Cottonseed oil soap	1000	1000	1000	500
								1.0% Cottonseed oil soap	2000	2000	1000	1000
12. (Tested at 37°C)				17.6	47.1% Pyro 35.3% Coconut oil soap			None	<200	<200	<200	<200
								5% Peptone				
								0.5% Cottonseed oil soap				
13.				15	15 30% Pyro 40% Coconut oil soap			None	1000	500	1000	1000
								None	<500	<500	500	500
									1000	500	<500	<500
14. (Tested at 37°C)			15	15	30% Pyro 40% Coconut oil soap			5% Peptone	<500	<500	<500	<500
								0.5% Cottonseed oil soap	2000	1000	500	<500
								None	1000	1000	<500	<500
15.	20		50		30% Sodium ..... 2-chloro-4 penyl penate			0.5% Cottonseed oil soap	<500	<500	<500	<500
									5000	5000	1500	<500
16.			50		20% Tripoly 10% tartaric acid			None	>500	>500	<500	<500
								0.5% Cottonseed oil soap	2000	2000	2000	2000
17.			75		25			None	<500	<500	<500	<500
								0.5% Cottonseed oil soap	2000	2000	2000	1000
18.			100					5% Peptone	1000	1000	1000	1000

perature in these studies varied from 18 to 25°C.

Table VII is a summary of tests where the testing solutions were maintained at 10°C. during the test period. Comparison of these data with similar formulations in Table V tested at 20°C. indicates that slightly greater concentrations of the proposed formulations are required at 10°C. than 20°C. The data suggests that the temperature coefficient between

Table VIa. Studies on the Disinfection of Stool by the Proposed Disinfectant****

500 ppm germicide	Result after exposure times as indicated* after 72 hours incubation at 37°C			
Exposure time	Lactose broth**		Plate count EMB***	
Liquid portion sampled	Sample		Sample	
5 min.	1	—	1	1
	2	—	2	1
	3	—	3	1
15 min.	1	Gr	1	4
	2	Gr	2	2
	3	Gr	3	1
30 min.	1	—	1	0
	2	—	2	3
	3	—	3	4
45 min.	1	Gr	1	3
	2	Gr	2	2
	3	Gr	3	4

*Sampled with 4 mm. 26 gauge pt. loop.
**No growth — growth, but no gas — growth with gas.
***EMB eosin methylene blue agar.
The lactose tubes showing gas were streaked onto EMB Agar. Colonies on EMB were not *E. coli*.
****Consists of a mixture of 75% sodium-chloro-phenyl phenate and 25% sodium-o-phenyl phenate.

Table VIb. Studies on the Disinfection of Stool by the Proposed Disinfectant*

1000 ppm germicide	Results after exposure times as indicated cultures incubated 72 hours at 37°C					
Exposure time	Lactose broth		Plate count EMB agar		Plate count TGE** agar	
Liquid portion sampled	Sample		Sample		Sample	
5 Min.	1	—	1	0	1	0
	2	—	2	0		
	3	—				
10 Min.	1	—	1	0	1	1
	2	—	2	0		
	3	—				
15 Min.	1	—	1	0	1	2
	2	—	2	0		
	3	—				
30 Min.	1	—	1	0	1	0
	2	—	2	0		
	3	—				
45 Min.	1	—	1	0	1	0
	2	—	2	0		
	3	—				
60 Min.	1	—	1	0	1	0
	2	—	2	0		
	3	—				
Stool samples						
10 Min.	1	Gr				
	2	Gr				
60 Min.	1	Gr				
	2	Gr				

*See footnotes for Table VIa.
**TGE Agar = Tryptone glucose extract agar, Bacto B2, Difco Co.

Table VIc. Studies on the Disinfection of Stool by the Proposed Disinfectant*

2000 ppm germicide		Results after exposure times as indicated cultures incubated 72 hours at 37°C		
Exposure time	Lactose broth		Plate count EMB agar	
Liquid portion samples	Sample		Sample	
5 min.	1	—	1	0
	2	—	2	0
	3	—		
10 min.	1	—	1	0
	2	—	2	0
	3	—		
20 min.	1	—	1	0
	2	—	2	0
	3	—		
30 min.	1	—	1	0
	2	—	2	0
	3	—		
45 min.	1	—	1	0
	2	—	2	0
	3	—		
60 min.	1	—	1	0
	2	—	2	0
	3	—		
Solid portion samples				
60 min.	1	Gr	1	12
	2	Gr	2	10
	3	Gr	3	9

*See footnotes for Table VIa.

Table VIII. Bactericidal Test on Samples of Proposed Disinfectant* Aged at 50°C for 540 Days

Sample tested and inactivator	PPM compound need to give zero counts in time (minutes) shown			
	2	4	6	8
Proposed disinfectant alone	<500	<500	<500	<500
Proposed disinfectant +5% peptone	<500	<500	<500	<500

*Proposed disinfectant is composed of 75% sodium chloro-o-phenyl phenate and 25% sodium-o-phenyl phenate.
Test organism: 22 — 26 hr. broth culture of E. coli, ATCC #26. Temp. of testing solution: 20 —0.2°C. Water for preparing solution: distilled water. Inactivator: 5% Bacto — Peptone where indicated. Subculture medium: Lecithin broth with 1.5% Bacto agar.

10°C. and 20°C. is roughly two or perhaps slightly less than two, when tested in the presence of five per cent peptone.

The data shown in Table VIII indicate that the proposed disinfectant formulation has almost full bactericidal activity against *E. coli* after 540 days storage at 50°C. The data suggests that this storage time and temperature do not appreciably affect the bacteriological effectiveness of the proposed disinfectant formulation, although visible darkening of the product was noted during the storage period.

Summary and Discussion

The conditions of use, as well as the manner in which the disinfectant is used, are important considerations in practical disinfection. The application of a concentrated dry-type disinfectant as a single agent for total disinfection of the contents of field latrine buckets appears to be impractical. The phenols in general act as coagulants and tend to reduce the organic matter concentration in solution by keeping the feces intact. In order to achieve complete disinfection of the stool by chemical means, it would probably be necessary to liquify the stool completely, then kill the organism. Liquifaction can be achieved by the use of fairly high concentration of caustic soda.

On the other hand, the danger of cross-contamination of the men who use the latrine bucket, as well as those cleaning the bucket, can be considerably reduced by disinfecting only the surface and liquified portions of the stools. In this connection, it is to be realized that in a pathological condition leading to diarrhea, relatively greater soil load will result, not only because of the liquified stool, but because of the poor absorption of food in the intestine.

On the basis of this study, 2,000 ppm of the proposed disinfectant will give satisfactory destruction of the bacteria in the liquid portion of the latrine bucket plus a reasonable margin of safety.

In order to substantiate further a use level of 2,000 ppm of the proposed disinfectant for field latrines and general purpose use, the phenol coefficient (3) and use-dilution confirmation tests (4) were carried out using the A.O.A.C. procedure. A phenol coefficient of 71 was found for the proposed disinfectant using *S. typhosa* as the test organism. The conventional method of arriving at the maximum safe use-dilution presumed to be equiv-

Table VII. Bactericidal Tests on the Proposed Disinfectant Against E. Coli at 10°C

Formulation	Conditions	PPM compound needed to give zero counts in time (minutes) shown below 2 min.	4 min.	6 min.	8 min.
25% sodium-o-phenyl phenate	alone	500	500	200	200
Plus 75% sodium chloro phenyl phenate	+5.0% Peptone	1000	1000	1000	1000
	+0.5% Soyabean oil soap	2000	2000	2000	2000
	+0.5% alkyl aryl sulfonate	2000	2000	2000	1000

Conditions of test: Test culture: *E. Coli*, Temperature of test solution: 10°C.

alent in efficiency to five per cent phenol is to multiply the phenol coefficient number found by the figure 20 to determine the number of parts of water in which one part of the disinfectant is to be incorporated. (3) A 1:1400 dilution of the proposed disinfectant was found necessary to kill *S. Cholerae suis* in the confirmation safe-use-dilution test. These confirmatory data very closely substantiate the phenol coefficient of 71, since (20 x 71 = 1420), a dilution of 1:1400 is, for practical purposes, the same as 1:1420 which is equivalent to 0.07% or 700 ppm. This data would then suggest that a concentration of 700 ppm of the proposed disinfectant would be an effective safe use concentration.

The results of the A.O.A.C. phenol coefficient and the use-dilution confirmation test indicate that a considerably lower concentration of the proposed disinfectant is required to kill the test organisms effectively, than is required to do an equivalent job using the modified phenol coefficient method, *E. coli* being the test organism. Since the modified phenol coefficient test is more severe than the standard A.O.A.C. test method, and one which would more nearly simulate the use conditions in a latrine bucket, a concentration of 2,000 ppm of the proposed disinfectant in the latrine bucket is suggested in order to maintain a reasonable margin of safety.

Current studies are being conducted on the use of 75 per cent sodium chloro-o-phenyl phenate and 25% sodium-o-phenyl phenate mixture as a general purpose disinfectant for walls, floors, etc. The results of these studies will be reported at a later date.

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